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SPONTANEOUS METAMORPHOSIS OF THE AMERICAN AXOLOTL

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THE following experiments on axolotl neoteny and metamorphosis are published, not because of the conclusive nature of the results obtained, but the reverse—because of their inconclusiveness. A record of the work seems warranted in order that other investigators of this problem may be spared considerable expense, time and effort due to unsuitability of the material for experimentation.

The latter part of April, 1922, one hundred and nine axolotl larvæ of *Amblystoma tigrinum* were received from Albuquerque, New Mexico. These animals were obtained through the courtesy of Mr. J. N. Gladding. They varied in length from four inches to fourteen inches, though the average total length was about seven inches. One animal measured fourteen inches from snout to tail tip, another measured eleven inches. They were the largest individuals of the lot. The animals were in excellent condition on arrival and none showed any indications of metamorphosis.

EXPERIMENT 1. AUTOPLASTIC THYROID TRANSPLANTATION

May 5, 1922, the thyroids of seven axolotls, seven inches in length, were removed under chloretone anesthesia and each gland transplanted intraperitoneally into the same individual from which it was taken. The idea was that the acquisition of a new blood and nerve supply by the gland in its new environment might permit the release of the accumulated secretion and so metamorphose the animal. It was shown by the writer ('21) that the thyroid glands of axolotls are highly active metamorphosis-inducing agents providing the hormone escapes into the

blood stream. In these forms there appears to be some inhibition of the secretory (excretory) functions of the thyroid, and the hormone is retained within the gland vesicles.

The experimental animals and their controls were kept in large aquaria with plenty of water and food. One of the grafted animals had metamorphosed by June 27. Three others transformed by July 1; a fifth animal died without transforming July 6. The two remaining axolotls had not metamorphosed by September 1. During the interval between May 21 and September 1, all of the controls spontaneously transformed. The experiment is, of course, without significance because of the unstable nature of the control material. It is highly probable that the operated animals would have metamorphosed just about as rapidly if the thyroid had been left in its normal position.

EXPERIMENT 2. HOMOPLASTIC THYROID TRANSPLANTATION

Five seven-inch axolotls were engrafted intraperitoneally with the thyroid gland of other animals of similar size and appropriately controlled by animals transplanted with pieces of muscle tissue.

The transplants were made May 2, 1922. One animal had transformed by June 3, a second by June 6, a third June 11. Two animals remained as larvæ and were re-engrafted June 11 with axolotl thyroids, and metamorphosed by July 3. In the meantime the controls also transformed. A large series of transplantation experiments were performed, using various endocrine glands, but in every case except two experiments the controls metamorphosed along with the operated individuals.

HETEROPLASTIC THYROID TRANSPLANTATION

Four eight-inch axolotls were engrafted intraperitoneally with the glandular tissue of adult *Necturus maculatus*. Each axolotl received the entire thyroid of a single

Necturus. The experiment was performed May 15. By June 11 all of the engrafted animals had transformed but none of the controls for this particular group, though normal, untreated animals used as checks for other experiments were metamorphosing during this interval.

Despite the unstable nature of the control material used, this experiment seems fairly sound and indicates that *Necturus* thyroids when injected in sufficient quantity will metamorphose axolotl. To be absolutely reliable this experiment should have been performed upon thyroidectomized forms, but unfortunately the unsuitable nature of the controls was not known until too late.

THYROID FEEDING EXPERIMENTS

Five six-inch axolotls were fed desiccated thyroid tissue (Parke, Davis and Company), containing 0.21 per cent. iodine by weight. The feeding was done by means of a pipette May 18. Two animals had transformed by May 27, and all by June 10. None of the controls metamorphosed during this interval but all transformed by July 25. The experiment seems trustworthy, especially in view of similar results obtained by other investigators on animals of the European strain.

HETEROPLASTIC PITUITARY TRANSPLANTATION

Five axolotls varying in length from four to seven inches were each grafted with two whole pituitary glands of adult *Rana clamata* frogs. The grafts were made May 5. June 3 one animal metamorphosed; June 7 a second transformed. June 10 the three remaining animals were reengrafted with frog pituitaries. All metamorphosed by June 25.

During the interval between May 5 and June 25 only two of the controls for this particular group transformed, but it must be remembered that control animals of other cultures were metamorphosing. The experiment is recorded for what it is worth, but the writer believes that

injection of fresh pituitary substance does induce axolotl metamorphosis possibly by serving to release the thyroid hormone. This experiment should be tried on the Mexican strain of axolotl which apparently rarely spontaneously metamorphoses and hence can be safely controlled.

THYROIDECTOMY AND METAMORPHOSIS

Eight axolotls varying from seven to fourteen inches were thyroidectomized and at the present writing, September 1, are still larvæ and show no indications of transforming. Out of the original one hundred and nine animals received from New Mexico these eight are the only ones that have not metamorphosed. It is a fairly safe assumption that these axolotls will remain permanently as larva now that the thyroid gland is lacking.¹

The thyroids of several animals were removed after the onset of metamorphosis, *i.e.*, after the tail fin and gills were undergoing reduction, but in all cases the removal of the thyroid failed to prevent the completion of metamorphosis.

DISCUSSION

The conclusion to be drawn from these experiments is that the New Mexican strain of axolotl is entirely too unstable to work with on any problem involving the methods of feeding, injection or transplantation, where the results require a lapse of several weeks to obtain. The animals can not be controlled when the thyroid apparatus is left intact. It is evident that conclusive experiments of the above kinds on the New Mexican strain of axolotl (where the animals themselves are used as

¹ The thyroidectomized animals were kept for five months and then injected with iodotyrosine and iodoserumglobulin. Metamorphosis resulted within a period of twenty days following injections of either substance. Two partially thyroidectomized animals which had failed to transform were metamorphosed by injection of iodoserumglobulin. Injections of tyrosin, dibromtyrosin and globulin had no effect upon metamorphosis. Uhlenhuth's conclusion that only thyroid iodine (iodine which has undergone transformation within the thyroid gland) is effective in metamorphosing urodele larvæ is invalid.

experimental material) can only be obtained by using thyroidectomized animals.

Professor Henry Laurens, of the Department of Physiology, informs me that several years ago he had a similar experience with axolotls from New Mexico. He received a shipment of several dozen in the spring, but was unable to prevent them from transforming shortly after arrival in New Haven. Only one animal of the lot failed to metamorphose and was kept two years in the laboratory, attaining a length of 14.25 inches. This individual was used by the writer for thyroid transplantation work.

The marked tendency of the New Mexican and other American axolotls to metamorphose spontaneously when moved from one locality to another prevents their being used for aquarium purposes. It is an odd fact that practically the only axolotls used as aquarium material in the United States are those that have been shipped from Europe.

The European strain seems to differ from the New Mexican form in regard to spontaneous metamorphosis, because these animals are handled by practically all aquarium dealers in Germany and can be obtained for a few cents apiece. Apparently they rarely spontaneously transform according to Jensen ('20), who has worked extensively with this strain. The curious thing about the New Mexican strain is that in their native habitat they too may remain for considerable periods as larva, yet when shipped from New Mexico to New Haven promptly metamorphose regardless of size or age. One large animal of this strain obtained by Professor Laurens failed to transform and was kept in the laboratory for two years; at the end of this time it showed no indications of metamorphosis and was killed for thyroid transplantation work.

According to Gadow ('08) the strain of axolotls established in Europe came originally from the vicinity of Mexico City. The first axolotls were brought to France by Marshal Forey in 1863, and the present strain is de-

scended from these animals. Gadow also states that the axolotls of Lake Xochimilco have never been known to metamorphose in their native habitat. However, several of the descendants of the animals taken to Europe did metamorphose, so that spontaneous transformation in the Mexican strain does sometimes occur, though rarely.

In an earlier paper ('22) the writer showed that the thyroid mechanism of axolotls is filled with physiologically active hormone capable of inducing metamorphosis but that the secretion is apparently not liberated into the blood stream, hence the retention of the larval characters despite the possession of a large well-formed gland. The thyroid of a fourteen-inch axolotl several years of age was extirpated and cut into small pieces, each piece then transplanted into an immature Anuran larva. The single axolotl thyroid promptly metamorphosed five such tadpoles within fourteen days, whereas left intact within the axolotl's body it was quite incapable of inducing transformation.

This same experiment was repeated upon thyroidectomized and hypophysectomized *Rana sylvatica* tadpoles with similar results. Small pieces of axolotl thyroid when engrafted into thyroidless and pituitaryless larvæ promptly induce metamorphosis within ten or twelve days.

It is quite clear from these experiments that axolotl neoteny is due to retention of the thyroid hormone within the gland vesicles. Under normal conditions and in its native habitat, the releasing mechanism apparently fails to act, but when the animals are shipped from one place to another and subjected to new environmental conditions metamorphosis promptly ensues. In the New Mexican strain slight stimulation is sufficient to initiate metamorphosis, but in the European and Mexican forms very powerful stimulation is needed to overcome the thyroid inhibition and release the secretion. In the European strain the following agents have been used successfully for inducing metamorphosis: thyroid feeding (Laufber-

ger '13), salicylic acid injections (Kaufman '18), iodine and iodoform injections (Hrischler '18-'19), organic iodine feeding—iodothyrosine, also injections of iodocasein, iodoserumglobulin and iodoserumalbumin (Jensen '21); and of course Marie von Chauvin's experiments are well known.

It is evident that the peculiar thyroid inhibition causing neoteny in axolotl is due to genetic factors and that the condition is hereditarily transmitted. It is interesting to note that in axolotl we have one of the best examples of hereditary transmission of an endocrine defect known. Attempts to explain neoteny by assuming that environmental agencies such as cold, altitude and the like are the chief causative factors are too crude to be seriously considered and for this reason—the aquarium dealers of Europe breed their animals as larvæ and the young grow up as axolotls, the matter of cold or altitude not entering into the question. As was previously mentioned, the European strain arose from a few animals taken to France in 1863.

Then, too, both Professor Laurens and myself received our animals from Albuquerque, New Mexico, where they breed. The animals were old when captured. The temperature of the pools in the vicinity of the city can not be very low even in winter—not nearly so cold as those of the middle western states, northern New York, Ohio, or Wisconsin—and axolotls have never been reported as occurring in these states so far as the writer is aware.

The *Amblystoma tigrinum* resulting from the metamorphosis of the axolotls during my experiments were placed in certain pools in the vicinity of New Haven where other species of *Amblystoma* are known to breed. The animals are full grown and should breed next spring (1923). By following the life history of the larvæ it is hoped that some new light may be shed upon the obscure and much debated problem of the relation of neoteny to environment.

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